FEBS Letters 583 (2009) 2720–2726

FEBS Letters

journal homepage: www.FEBSLetters.org

# Hepatitis C virus NS5A protein increases hepatic lipid accumulation via induction of activation and expression of PPARgamma

KyeongJin Kim [a], Kook Hwan Kim [a], Eunsin Ha [a], Jin Young Park [a], Naoya Sakamoto [b], JaeHun Cheong [a,*]

[a] Department of Molecular Biology, College of Natural Sciences, Pusan National University, Busan 609-735, Republic of Korea
[b] Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo 113-8519, Japan

ARTICLE INFO

*Article history:*
Received 11 July 2009
Accepted 17 July 2009
Available online 23 July 2009

Edited by Hans-Dieter Klenk

*Keywords:*
Hepatitis C virus
NS5A
Lipid accumulation
PGC-1
PPARγ

ABSTRACT

**Steatosis is an established risk factor for disease progression in cases of chronic hepatitis C. Recently it was demonstrated that Hepatitis C virus (HCV) core and non-structural (NS) 2 proteins (NS2) induce lipid accumulation in hepatic cells. However, it has yet to be determined whether other HCV proteins are associated with lipid metabolism. The NS5A augmented the transcriptional activity and gene expression of PPARγ. Furthermore, NS5A increased the ability to recruit the transcriptional coactivator PGC-1s to the PPRE with PPARγ, as well as the interaction with PPARγ2 and PGC-1α. Our results indicate that NS5A may exploit multiple strategies that enhance PPARγ-induced lipid accumulation.**

*Structured summary:*
MINT-7229685: *PPAR gamma 2* (uniprotkb:P37231-2) *physically interacts* (MI:0914) with *PGC1 alpha* (uniprotkb:Q9UBK2) by *pull down* (MI:0096)
MINT-7229712: *PPAR gamma 2* (uniprotkb:P37231-2) *physically interacts* (MI:0914) with *NS5A* (uniprotkb:P26662) by *pull down* (MI:0096)
MINT-7229698: *PPAR gamma 2* (uniprotkb:P37231-2) *physically interacts* (MI:0914) with *PGC1 alpha* (uniprotkb:Q9UBK2) by *anti tag coimmunoprecipitation* (MI:0007)
MINT-7229731: *PPAR gamma 2* (uniprotkb:P37231-2) *physically interacts* (MI:0914) with *NS5A* (uniprotkb:P26662) by *anti tag coimmunoprecipitation* (MI:0007)



## 1. Introduction

Hepatitis C virus (HCV) is classified into the genus *Hepacivirus* of the family *Flaviviridae* [1]. As the case with all the members of this family, HCV is an enveloped, single-stranded, and positive-sense RNA virus. Upon translation, HCV polyprotein is proteolytically processed by both cellular and viral proteases into at least 10 individual proteins, including four structural proteins (core, E1, E2 and p7) and six NS proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [2]. As the penultimate protein processed from HCV polyprotein precursor, NS5A is a proline-rich hydrophilic phosphoprotein and may exist in dimeric form [3]. Although no intrinsic enzymatic activity has yet been ascribed to NS5A, it likely functions via interactions with other NS proteins and host cell factors [2].

Peroxisome proliferators-activated receptors (PPARs) are ligand-activated nuclear receptors belonging to the steroid/thyroid hormone receptor superfamily; 3 isoforms designated as α, β/δ, and γ exist, all of which are known to be involved in lipid homeostasis. Increased PPARγ expression has been reported in high-fat diet-induced liver steatosis [4].

Chronic HCV infection may result in a wide clinical and prognostic spectrum of severity and progression rates in cases of liver disease, ranging from chronic hepatitis to cirrhosis and hepatocellular carcinoma [5]. Hepatic steatosis is detected in almost 50% of HCV-infected patients, which suggests that it is a crucial contributor to nonalcoholic fatty liver disease (NAFLD) [6], a severe and progressive liver disease resulting in the development of cirrhosis [7]. Recently, it has been reported that HCV core proteins increase hepatic lipid accumulation via the activation of SREBP-1 and PPARγ [8], and that NS2 can upregulate the transcription of SREBP-1c and fatty acid synthase (FAS) [9]. However, the precise molecular mechanisms underlying HCV NS5A-associated steatosis have yet to be clearly characterized. In this study, we explored the possibility that NS5A induces lipid accumulation in hepatocytes and the activity and expression of PPARγ may be deregulated, therefore playing roles in NS5A-induced hepatic triglycerides deposits. Our

* Corresponding author. Fax: +82 51 513 9258.
*E-mail addresses:* molecule85@pusan.ac.kr, jhcheong2@lycos.co.kr (J. Cheong).

0014-5793/$36.00 
doi:10.1016/j.febslet.2009.07.034

results indicate that NS5A-mediated deregulation of PPARγ may be involved in HCV-induced fatty liver disease.

## 2. Materials and methods

### 2.1. Plasmids, reagents, and antibodies

pNK/Flag/NS5A, pcDNA4/myc/PGC-1α, and pcDNA3/Flag/PGC-1β were generously donated by Dr. Seishi Murakami, Dr. Toren Finkel, and Dr. Bruce M. Spiegelman [10–12]. pcDNA3/HA/PPARγ, pcDNA3/GFP/PPARγ, pcDNA3/GST/PPARγ, pcDNA3/HA/PGC-1α, pM/PGC-1α, pVP16/PGC-1α, and pcDNA3/HA/NS5A constructs were subcloned via the insertion of the PCR fragments of ORF into pcDNA3/HA, pM (Gal4), pVP16 (Clontech), or pcDNA3/GFP [13,14]. Other constructs were all described previously [8,13].

Rosiglitazone and Ciglitazone were obtained from Cayman. The transfection reagent PolyFect and JetPEI were purchased from QIAGEN and Polyplus-transfection. All other reagents were purchased from Sigma. The antibodies against PPARγ, GST, and GFP were purchased from Santa Cruz Biotechnology Inc. and Actin, Flag, and HA-antibody was obtained from Sigma, Cell Signaling, and Roche, respectively. The anti-NS5A polyclonal antibody was kindly gifted from Dr. Soon B. Hwang (Hallym University, Korea).

### 2.2. Cell culture and transient ransfection

Chang liver, HepG2, and Huh7 cell lines were maintained in DMEM–10% fetal bovine serum (FBS; Abclone). Transient transfections were conducted using PolyFect or JetPEI with the indicated reporter plasmids and mammalian expression vectors. Total amounts of expression vectors were maintained at constant levels via the addition of empty vectors. Relative luciferase activities were measured with luciferin (BD Biosciences).

### 2.3. Establishment of Chang liver cells expressing NS5A proteins

Chang liver cells were transfected with 2 μg of pcDNA3/HA or pcDNA3/HA/NS5A using JetPEI reagents in accordance with the manufacturer's instructions. After 48 h, the cells were grown in a medium containing 800 μg/ml G418. Following 2 weeks of selection, sorted single cells were grown under an additional 2 weeks of selection and expanded into stable cells. The candidate clones were analyzed via Western blotting using specific HA-antibody.

### 2.4. RNA isolation and RT-PCR

Total RNA was prepared using TRIzol (Invitrogen) in accordance with the manufacturer's recommendations. The cDNA was synthesized from 3 μg of total RNA with Moloney murine leukemia virus (MMLV) Reverse Transcriptase (Promega) using a random hexamer (Cosmo, Korea) for 1 h at 37 °C. A one-twenty fifth aliquot of the cDNA was subjected to PCR amplification using gene-specific primers. The PCR primers for PPARγ gene amplification were: 5′-GAAATGACCATGGTTGAC-3′ (sense), 5′-GATGCAGGCTCCACTTTG-3′ (antisense); for NS5A amplification: 5′-TAGCAGTGCTCACTTCCATGCTCA-3′ (sense), 5′-AGGATCTCCGCCGCAATGGATATT-3′ (antisense); for β-actin gene amplification: 5′-GACTACCTCATGAAGATC-3′ (sense), and 5′-GATCCACATCTGCTGGAA-3′ (antisense).

### 2.5. Establishment of Huh7 cells expressing HCV replicons and interferon-cured cells

An HCV subgenomic replicon plasmid, pRep-Feo, was derived from pRep-Neo (originally referred to as pHCVIbneo-delS) [15]. The pRep-Feo expressed a fusion gene comprised of *firefly* luciferase (Fluc) and neomycin phosphotransferase, as described else-

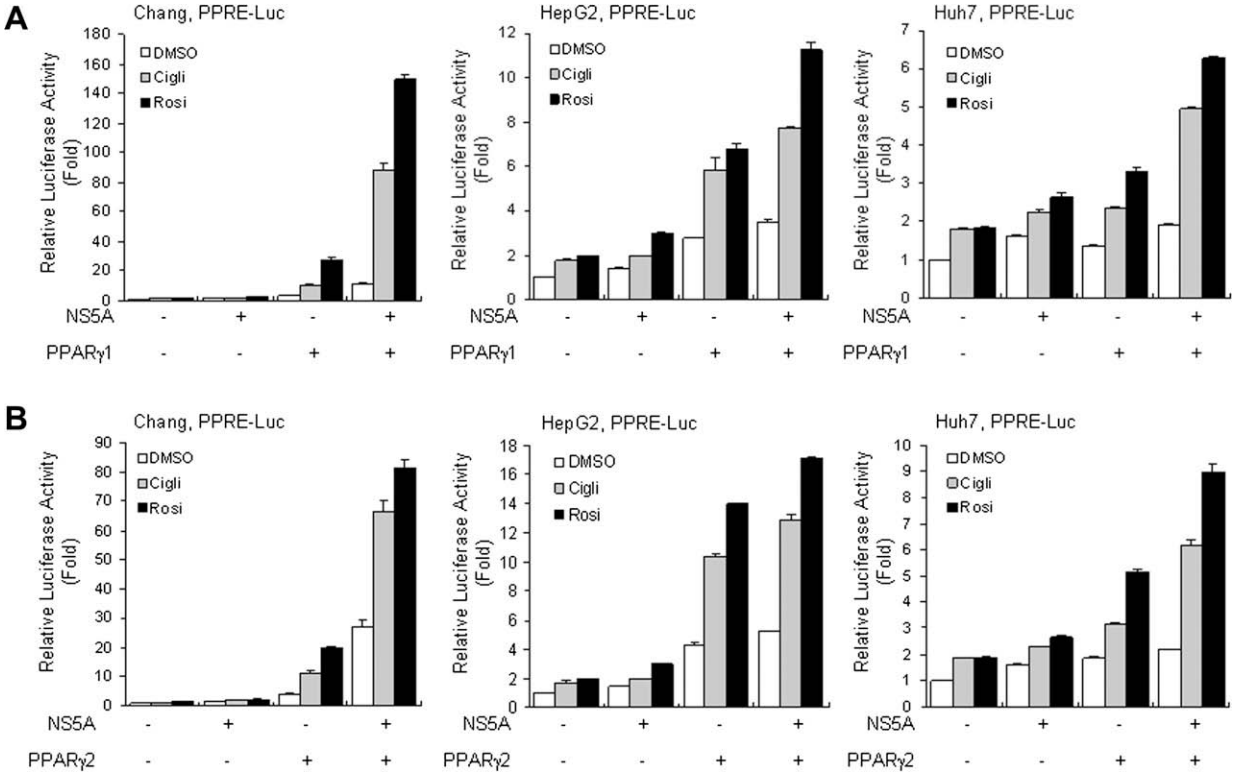


**Fig. 1.** NS5A induces the transcriptional activity of PPARγ in hepatocytes. (A) PPRE-tk-Luc was cotransfected with the expression vectors for PPARγ1 and NS5A into Chang liver, HepG2, and Huh7 cells. Transfected cells were incubated in the presence or absence of 10 μM Ciglitazone and Rosiglitazone. (B) Cells were cotransfected with a PPRE-tk-luciferase reporter, PPARγ2, and NS5A. Transfected cells were incubated for 24 h in the presence or absence of 10 μM Ciglitazone and Rosiglitazone. Luciferase activity was measured and values are expressed as means ± S.D. for at least two or more independent experiments.

where. Replicon RNA was synthesized in vitro using T7-RNA polymerase and transfected into the Huh7 cells via electroporation. After culturing in the presence of G418, the cell lines stably expressing the replicons were established and designated Huh7/Rep-Feo. To establish interferon-alpha (IFN-α)-cured cells, HCV replicon cells were treated with $10^3$ U/ml IFN-α (PBL Biomedical Laboratories) for 10 days. The expression of HCV replicon was confirmed by RT-PCR and Western blotting.

### 2.6. In vivo interaction assays

Forty-eight hours after transfection, the cells were solubilized with 300 μl of radioimmunoprecipitation assay (RIPA) buffer. The cleared lysates were mixed with 40 μl of glutathione-sepharose beads and rotated overnight at 4 °C. The bound proteins were eluted in 15 mM reduced glutathione, separated via SDS–PAGE, and transferred to polyvinylidene difluoride (PVDF) membranes for blotting.

### 2.7. Coimmunoprecipitation

For the immunoprecipitations, cells were plated and transfected as indicated plasmids. Cells were lysed via the addition of RIPA buffer and an aliquot of the lysate was removed for the input control, and the remainder was immunoprecipitated overnight with the specific antibody and protein G-Sepharose (50% suspension; Invitrogen). The lysates and immunoprecipitates were separated via SDS–PAGE and transferred onto PVDF membranes for blotting.

### 2.8. Oil Red O staining

ORO staining was conducted in accordance with the previously described procedure, with minor modifications [14]. Cells were stained overnight in freshly diluted ORO solution. The stain was then removed, and the cells were washed twice with water and absorbance of eluted ORO by adding 100% isopropanol at 500 nm was measured in a spectrophotometer.

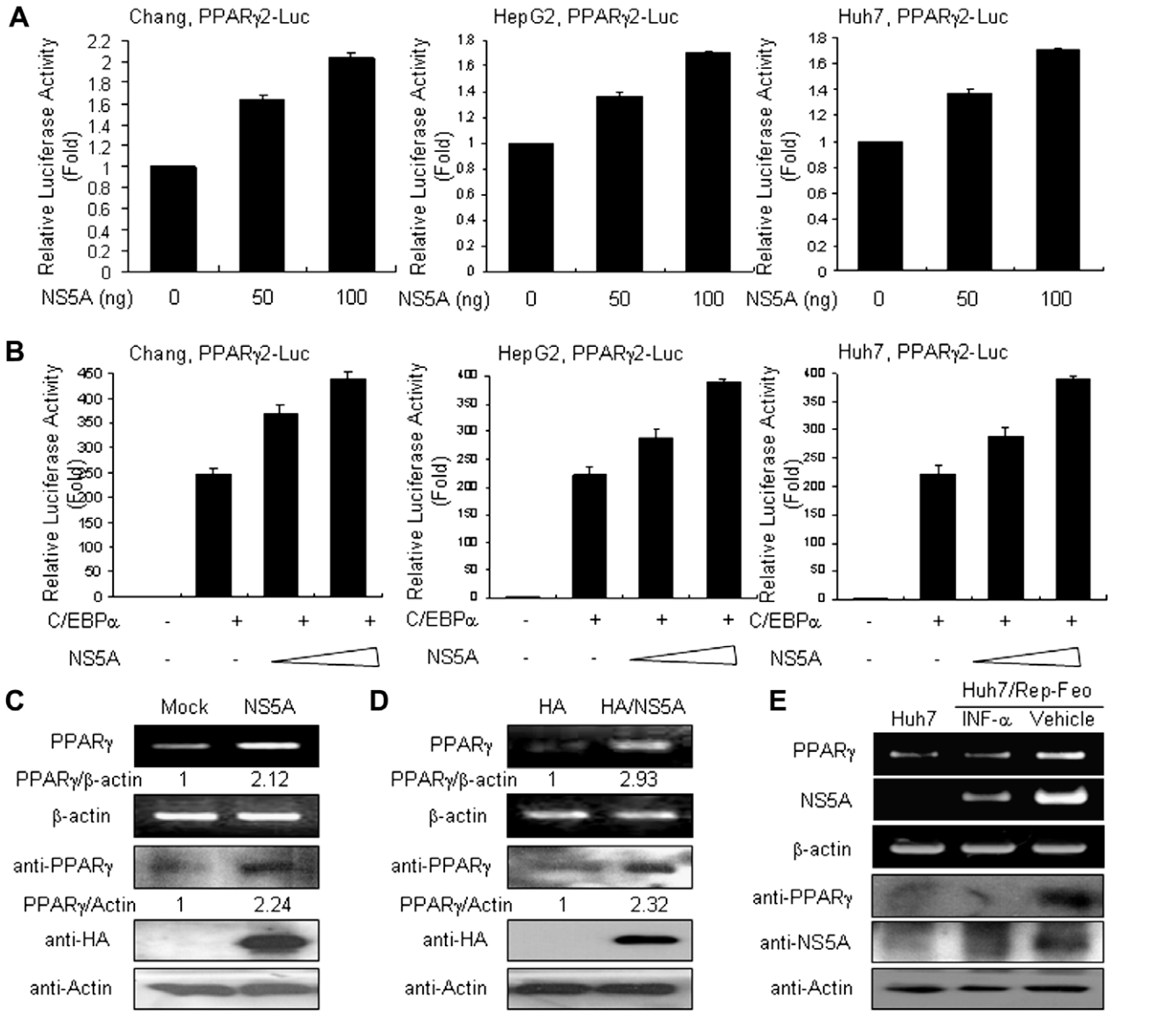


**Fig. 2.** NS5A promotes PPARγ mRNA and protein expression. (A) Chang liver, HepG2, and Huh7 cells expressing NS5A (50 and 100 ng) were assayed for their ability to transactivate a PPARγ2 promoter. (B) Cells were transfected with indicated expression plasmid. After 48 h of transfection, luciferase activity was determined. (C) The increase of PPARγ gene expression in transiently NS5A-transfected Chang liver cells. RT-PCR and Western blotting were performed as indicated. (D) The enhanced expression of PPARγ gene in Chang/HA/NS5A stable cells. Western blotting and RT-PCR were performed. (E) Total cell lysates or RNAs were harvested from Huh7, IFN-α-cured and HCV subgenomic replicon cells. RT-PCR and immunoblotting were performed as indicated.

### 2.9. RNA interference and transfection

For the siRNA-mediated downregulation of PPARγ, PPARγ-specific siRNA and negative control siRNA were purchased from Bioneer (Daejeon, Korea). Chang liver cells were transfected with either the siRNA molecule specific for PPARγ or a negative control siRNA using HiPerFect reagent (QIAGEN).

### 2.10. Statistical analysis

Statistical analyses were carried out by unpaired or paired *t*-test as appropriate. All data are reported as means ± S.D. *P* value of <0.05 was considered significant.

## 3. Results

### 3.1. HCV NS5A induces the transcriptional activity of PPARγ

Chronic hepatitis C (CHC) is associated frequently with hepatic steatosis and PPARγ cultivates hepatic steatosis. We investigated the effect of nine individual proteins of HCV (core191, core-glycoprotein E1 fusion protein, E2, and six NS proteins, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) on PPARγ transactivation. In order to determine whether nine proteins affect the transcriptional activity of PPARγ, we cotransfected a reporter plasmid harboring multimerized PPAR binding sites with the expression plasmid of PPARγ1, PPARγ2, and nine proteins. In particular, core191 and NA5A significantly affected the transcriptional activity of PPARγ1 and PPARγ2. Previously, we reported that core191 increases hepatic lipid accumulation by SREBP1 and PPARγ activation [8].

As shown in Fig. 1A and B, NS5A profoundly augmented the activation of reporter gene expression by PPARγ1 (Fig. 1A) and PPARγ2 (Fig. 1B) in the presence or absence of two synthetic PPARγ agonists, Ciglitazone and Rosiglitazone, in a variety of hepatic cell lines. These results indicate that NS5A protein is an important regulator in HCV-induced PPARγ activation.

### 3.2. HCV NS5A enhances the mRNA and protein expression of PPARγ

It might be expected that the increased PPARγ activity induced by NS5A is accompanied by increases in the levels of its gene expression. We found that NS5A augmented the promoter activity of PPARγ2 gene as a dose-dependent manner, as shown in comparisons of luciferase activity with basal levels (Fig. 2A). Since C/EBPα has been reported to upregulate PPARγ expression [16], we investigated the PPARγ2-luciferase reporter activity with cotransfection

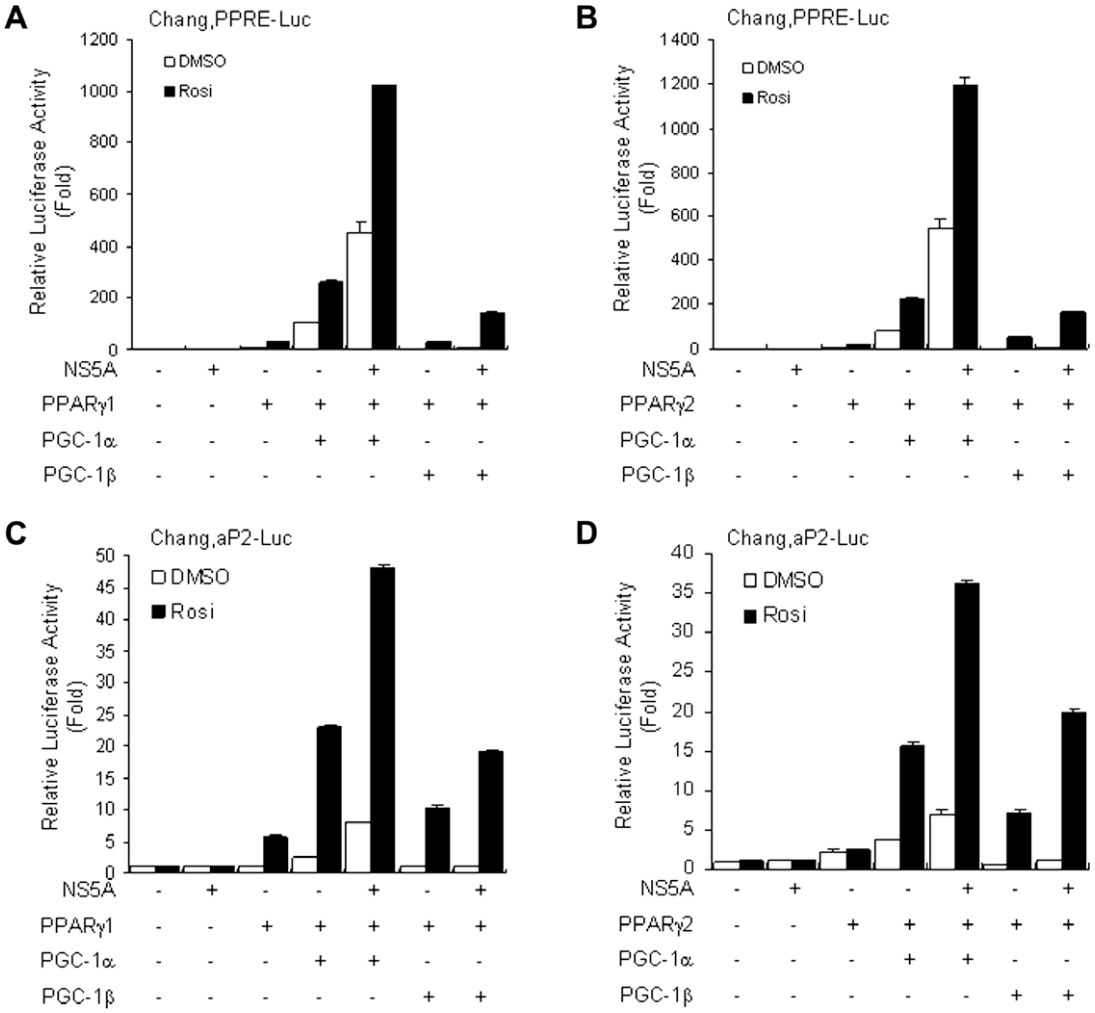


**Fig. 3.** PGC-1s potentiate the PPARγ-mediated transactivation with NS5A. (A and B) Chang liver cells were transiently transfected with PPRE-tk-Luc construct, in combination with PPARγ1 (A) or PPARγ2 (B) and NS5A in the presence or absence of PGC-1α and PGC-1β transfection. Cells were treated with 10 μM Rosiglitazone or vehicle (DMSO) for 24 h. (C and D) Chang liver cells expressing PPARγ1 (C), or PPARγ2 (D), NS5A, PGC-1α, and PGC-1β were assayed for their ability to transactivate the aP2 promoter. Cells were treated with 10 μM Rosiglitazone or vehicle for 24 h. Luciferase activity was measured.

of C/EBPα and NS5A expression plasmids. A reporter assay indicated that NS5A increased the activity of PPARγ2 promoter in cooperation with C/EBPα as a dose-dependent manner (Fig. 2B).

Furthermore, as shown in Fig. 2C, the mRNA and protein induction in RT-PCR and Western blotting were confirmed for PPARγ expression by transiently transfected NS5A. In addition, to better assess the role of NS5A protein in PPARγ expression, we established Chang liver cells evidencing stable expression of HA-tagged NS5A proteins, and confirmed the expression by immunoblotting with the HA-antibody. We investigated that PPARγ protein and mRNA expression were significantly elevated in Chang/HA/NS5A stable cells (Fig. 2D).

To further investigate whether the expression of PPARγ was regulated by viral protein in the context of HCV RNA replication, Huh7/Rep-Feo cells were treated with IFN-α for 10 days. The mRNA and protein expression levels of PPARγ were significantly elevated in HCV subgenomic replicon cells as compared with Huh7 or the IFN-α-cured cells.

### 3.3. HCV NS5A increases the transactivation of PGC-1s

PGC-1 was initially identified as a PPARγ-interacting protein from a BAT library, and was subsequently shown to associate with an array of nuclear receptors (NRs) and transcription factors [17,18]. Initially, the cotransfection of PGC-1α and PGC-1β with PPARγ1 (Fig. 3A) or PPARγ2 (Fig. 3B) in the presence of NS5A expression further stimulated transactivity of the PPRE promoter. In particular, PGC-1α increased PPARγ1 and PPARγ2 activity more in the presence of NS5A as compared to PGC-1β. We additionally assessed the effects on the native promoter activity of adipocyte fatty-acid-binding protein, aP2 (encoded by *Ap2*), a member of the intracellular fatty-acid-binding protein (FABP) family, which is an intact target of PPARγ in adipose cells [19]. Although aP2 genes predominantly are not expressed in liver, we exploited the overexpression of the aP2 promoter. As shown in Fig. 3C and D, the cotransfection of PGC-1α and PGC-1β with PPARγ1 (Fig. 3C) or PPARγ2 (Fig. 3D) and NS5A further elevated transcription from the native promoters of aP2.

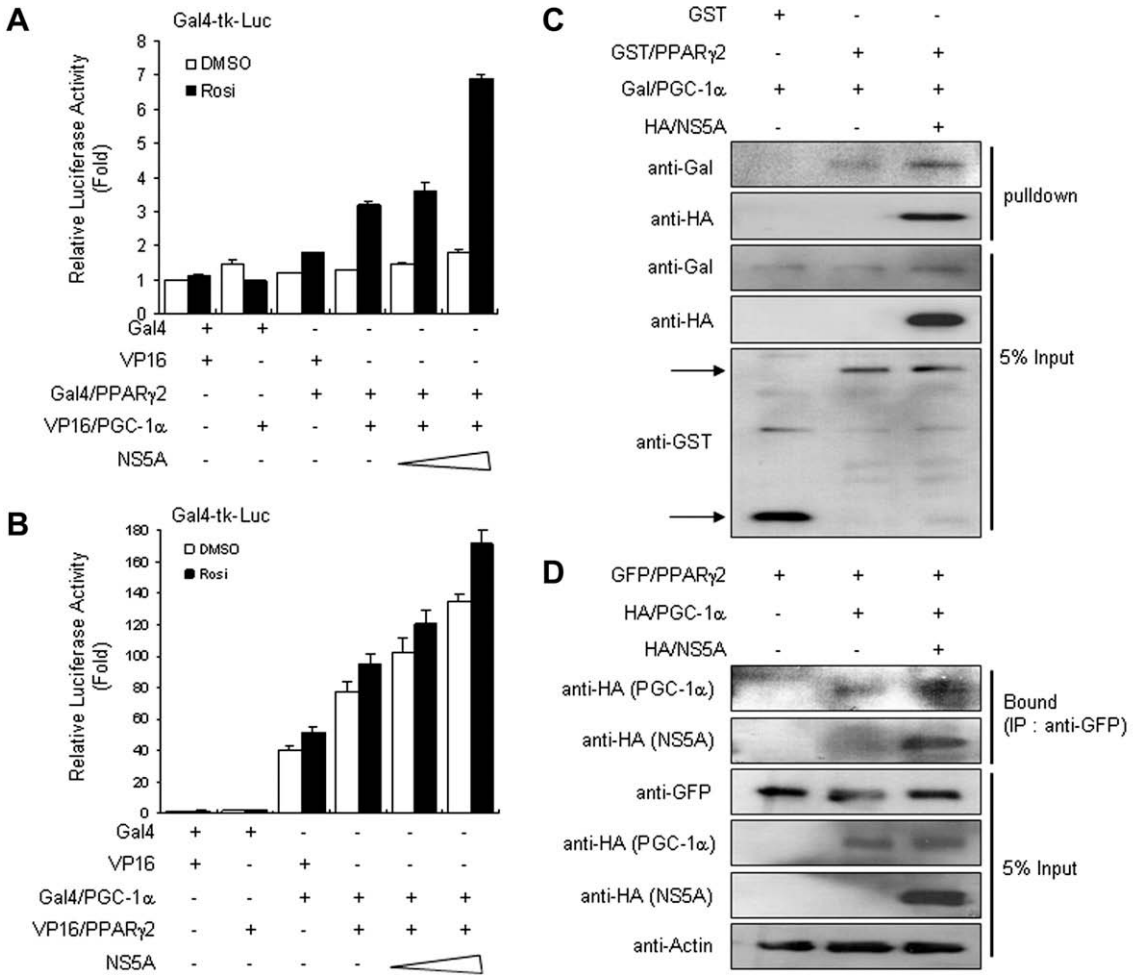


**Fig. 4.** NS5A enhances the interaction with PPARγ2 and PGC-1α. (A) Chang liver cells were cotransfected with expression vectors encoding Gal4/PPARγ2, VP16/PGC-1α, NS5A, and the reporter construct Gal4-tk-Luc. Cells were grown in the absence or presence of 10 μM Rosiglitazone. The histogram represents the transcriptional activity of the various Gal4/DBD/PPARγ fusion proteins. (B) Chang liver cells were cotransfected with expression vectors encoding Gal4/PGC-1α, VP16/PPARγ2, NS5A, and the reporter construct Gal4-tk-Luc. Cells were grown 24 h in the absence or presence of 10 μM Rosiglitazone. The luciferase assay was performed. (C) Chang liver cells were transfected with expression vectors for Gal/PGC-1α, HA/NS5A, GST/PPARγ2, and GST empty vector. Whole cell lysates were incubated with GST or GST/PPARγ2 fusion proteins bound to glutathione-sepharose 4B bead and bounded proteins were analyzed by Western blot. (D) Chang liver cells were transfected with plasmids as indicated. Total lysates from transfected cells were subjected to immunoprecipitation using antibodies specific for the GFP-epitope tag. Both lysates and precipitates were analyzed by immunoblotting with antibodies specific for HA.

### 3.4. HCV NS5A induces the recruitments of PGC-1α

Above results prompted us to attempt to investigate using various constructs whether the interaction between the PPARγ and PGC-1α enhances by NS5A proteins. We exploited the mammalian two hybrid system. It was observed that the interaction between Gal4-fused PPARγ2 and VP16-fused PGC-1α was enhanced by NS5A protein as a dose-dependent manner (Fig. 4A). Furthermore, the reverse attempt using Gal4/PGC-1α and VP16/PPARγ2 showed effects similar to those observed in Fig. 5A (Fig. 4B).

In an effort to determine whether NS5A enhances the interaction between PPARγ2 and PGC-1α on the cellular level, we also employed other approaches. Chang liver cells were transfected with constructs encoding for the GST-fused PPARγ2, Gal-tagged PGC-1α, and HA-tagged NS5A as indicated. As shown in Fig. 4C, the results of in vivo GST pulldown indicated that PGC-1α only interacted with GST-PPARγ2 and not GST proteins. Also, NS5A significantly enhanced the interaction between two proteins. We observed similar results using coimmunoprecipitation using anti-GFP antibodies. The GFP-tagged PPARγ2 was bound to HA-tagged PGC-1α and expression of NS5A enhanced the interaction of two proteins (Fig. 4D). Collectively, these findings indicate that NS5A performs a critical function in the formation of an active transcriptional complex of PPARγ2 and PGC-1α for PPARγ activation.

### 3.5. PPARγ is necessary for HCV NS5A-induced hepatic lipid accumulation

In order to examine the effects of NS5A protein in the lipid accumulation, Chang/HA/NS5A stable cells and parent Chang/HA stable cells were stained with ORO. The percentage of ORO-positive cells of Chang liver/HA/NS5A stable clone were significantly higher than in Chang/HA stable cells (Fig. 5A). Also, in an effort to determine whether PPARγ performs a function in NS5A-induced lipid accumulation, we attempted to knockdown PPARγ expression using siRNA specific for PPARγ. Chang liver cells were cotransfected with the NS5A construct and/or siPPARγ. As shown in Fig. 5B, siPPARγ-transfected cells did not affect hepatic lipid accumulation, even in the presence of NS5A. These results clearly indicate that PPARγ is a crucial transcription factor which mediates NS5A-induced lipid accumulation.

## 4. Discussion

HCV infects >170 million individuals worldwide and causes liver disease, including hepatic steatosis, cirrhosis, and eventually hepatocellular carcinoma (HCC) [20]. The reported prevalence of steatosis in patients with CHC varies between 40% and 80%, depending on the features of the population studied with regard to alcohol consumption, the prevalence of overweight/obesity, diabetes, and other risk factors of fatty liver [21]. HCV core protein expression has been demonstrated to activate various pathways of lipid metabolism [8,22,23]. However, the precise functions of other proteins among HCV proteins in the development of fatty liver remain to be determined.

NS5A interferes with the response to interferon and appears to perform a crucial role in viral replication [24]. Also, NS5A augments the anti-apoptotic effect of bcl-2 and inhibits the pro-apoptotic action of bax [25]. However, other functions of NS5A have yet to be clearly defined. In this report, our results provide the first evidence suggesting that NS5A protein can increase hepatic lipid accumulation by inducing an increase in the expression and activity of PPARγ.

It is well established that modulation of PPAR activity maintains cellular and whole-body glucose and lipid homeostasis. Recent studies have established a role for hepatic PPARγ in the development of hepatic steatosis in mouse model. According to these studies, hepatic PPARγ is able to contribute to hepatic steatosis through increasing hepatic triglyceride production and hepatic lipid uptake [26,27].

In conclusion, we have determined that NS5A augments hepatic PPARγ activation and expression. It has been demonstrated that NS5A enhances the recruitment of PGC-1α to PPARγ via the enhancement of interaction between these proteins. Hence, the results regarding NS5A pave the way for an important function as a selective target of PPARγ coactivation circuitry. Notably, we deter-

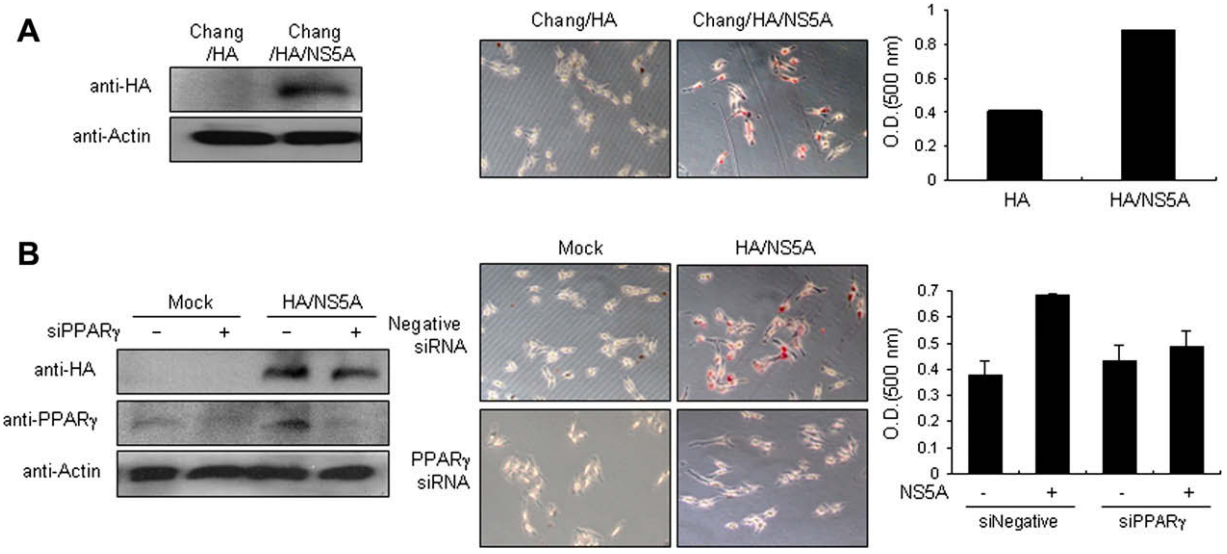


**Fig. 5.** HCV NS5A increases hepatic lipid accumulation. (A) The effect of expression of NS5A proteins in hepatic lipid accumulation. The expression levels of NS5A protein in Chang/HA/NS5A stable cells were analyzed by Western blotting using HA antibodies. ORO staining reveals the increased hepatic lipids in Chang/HA/NS5A stable cells compared with parent stable cells. (B) The effect of PPARγ in NS5A-induced lipid accumulation. For the siRNA-mediated downregulation of PPARγ, negative control siRNA or PPARγ-specific siRNA was transfected with or without NS5A proteins into Chang liver cells. The transfected cells were analyzed by Western blotting. The siPPARγ were transfected with or without NS5A into Chang liver cells. Cells were stained with ORO and photographed. The absorbance of eluted ORO was determined at 500 nm.

mined that NS5A ameliorates hepatic lipid accumulation via the regulation of PPARγ and may contribute to HCV-induced hepatic steatosis.

## Acknowledgments

This study was supported by a grant from the National R&D Program for Cancer Control, Ministry of Health & Welfare, Republic of Korea (0820140). The authors wish to thank Dr. Seishi Murakami, Dr. Toren Finkel, Dr. Bruce M. Spiegelman, Dr. Soon B. Hwang for pNK/Flag/NS5A and pcDNA4/myc/PGC-1α, pcDNA3/Flag/PGC-1β vectors, NS5A antibodies.

## References

[1] Reed, K.E. and Rice, C.M. (2000) Overview of hepatitis C virus genome structure, polyprotein processing, and protein properties. Curr. Top. Microbiol. Immunol. 242, 55–84.
[2] Huang, Y., Staschke, K., De Francesco, R. and Tan, S.L. (2007) Phosphorylation of hepatitis C virus NS5A nonstructural protein: a new paradigm for phosphorylation-dependent viral RNA replication? Virology 364, 1–9.
[3] Tellinghuisen, T.L., Marcotrigiano, J. and Rice, C.M. (2005) Structure of the zinc-binding domain of an essential component of the hepatitis C virus replicase. Nature 435, 374–379.
[4] Inoue, M. et al. (2005) Increased expression of PPARgamma in high fat diet-induced liver steatosis in mice. Biochem. Biophys. Res. Commun. 336, 215–222.
[5] Adinolfi, L.E., Gambardella, M., Andreana, A., Tripodi, M.F., Utili, R. and Ruggiero, G. (2001) Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology 33, 1358–1364.
[6] Ramesh, S. and Sanyal, A.J. (2004) Hepatitis C and nonalcoholic fatty liver disease. Semin. Liver Dis. 24, 399–413.
[7] Bacon, B.R., Farahvash, M.J., Janney, C.G. and Neuschwander-Tetri, B.A. (1994) Nonalcoholic steatohepatitis: an expanded clinical entity. Gastroenterology 107, 1103–1109.
[8] Kim, K.H., Hong, S.P., Kim, K., Park, M.J., Kim, K.J. and Cheong, J. (2007) HCV core protein induces hepatic lipid accumulation by activating SREBP1 and PPARgamma. Biochem. Biophys. Res. Commun. 355, 883–888.
[9] Oem, J.K., Jackel-Cram, C., Li, Y.P., Zhou, Y., Zhong, J., Shimano, H., Babiuk, L.A. and Liu, Q. (2008) Activation of sterol regulatory element-binding protein 1c and fatty acid synthase transcription by hepatitis C virus non-structural protein 2. J. Gen. Virol. 89, 1225–1230.
[10] Ichida, M., Nemoto, S. and Finkel, T. (2002) Identification of a specific molecular repressor of the peroxisome proliferator-activated receptor gamma Coactivator-1 alpha (PGC-1alpha). J. Biol. Chem. 277, 50991–50995.
[11] Lin, J., Puigserver, P., Donovan, J., Tarr, P. and Spiegelman, B.M. (2002) Peroxisome proliferator-activated receptor gamma coactivator 1beta (PGC-1beta), a novel PGC-1-related transcription coactivator associated with host cell factor. J. Biol. Chem. 277, 1645–1648.
[12] Yamashita, T., Kaneko, S., Shirota, Y., Qin, W., Nomura, T., Kobayashi, K. and Murakami, S. (1998) RNA-dependent RNA polymerase activity of the soluble recombinant hepatitis C virus NS5B protein truncated at the C-terminal region. J. Biol. Chem. 273, 15479–15486.
[13] Kim, K.H. et al. (2007) Hepatitis B virus X protein induces hepatic steatosis via transcriptional activation of SREBP1 and PPARgamma. Gastroenterology 132, 1955–1967.
[14] Kim, K., Kim, K.H., Kim, H.H. and Cheong, J. (2008) Hepatitis B virus X protein induces lipogenic transcription factor SREBP1 and fatty acid synthase through the activation of nuclear receptor LXR alpha. Biochem J. 416, 219–230.
[15] Yokota, T. et al. (2003) Inhibition of intracellular hepatitis C virus replication by synthetic and vector-derived small interfering RNAs. EMBO Rep. 4, 602–608.
[16] Rosen, E.D., Hsu, C.H., Wang, X., Sakai, S., Freeman, M.W., Gonzalez, F.J. and Spiegelman, B.M. (2002) C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. Genes Dev. 16, 22–26.
[17] Puigserver, P., Wu, Z., Park, C.W., Graves, R., Wright, M. and Spiegelman, B.M. (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92, 829–839.
[18] Wu, Z. et al. (1999) Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98, 115–124.
[19] Tontonoz, P., Hu, E. and Spiegelman, B.M. (1995) Regulation of adipocyte gene expression and differentiation by peroxisome proliferator activated receptor gamma. Curr. Opin. Genet. Dev. 5, 571–576.
[20] Wasley, A. and Alter, M.J. (2000) Epidemiology of hepatitis C: geographic differences and temporal trends. Semin. Liver Dis. 20, 1–16.
[21] Asselah, T., Rubbia-Brandt, L., Marcellin, P. and Negro, F. (2006) Steatosis in chronic hepatitis C: why does it really matter? Gut 55, 123–130.
[22] Hope, R.G. and McLauchlan, J. (2000) Sequence motifs required for lipid droplet association and protein stability are unique to the hepatitis C virus core protein. J. Gen. Virol. 81, 1913–1925.
[23] Barba, G. et al. (1997) Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets. Proc. Natl. Acad. Sci. USA 94, 1200–1205.
[24] Bartenschlager, R. (2006) Hepatitis C virus molecular clones: from cDNA to infectious virus particles in cell culture. Curr. Opin. Microbiol. 9, 416–422.
[25] Wang, J. et al. (2006) Hepatitis C virus non-structural protein NS5A interacts with FKBP38 and inhibits apoptosis in Huh7 hepatoma cells. FEBS Lett. 580, 4392–4400.
[26] Gavrilova, O. et al. (2003) Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass. J. Biol. Chem. 278, 34268–34276.
[27] Yu, S. et al. (2003) Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor gamma1 (PPARgamma1) overexpression. J. Biol. Chem. 278, 498–505.